

# XAFS and X-Ray and Electron Microscopy Investigations of Radionuclide Transformations at the Mineral-Microbe Interface

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**Abstract.** The microenvironment at and adjacent to surfaces of actively metabolizing cells, whether in a planktonic state or adhered to mineral surfaces, can be significantly different from the bulk environment. Microbial polymers (polysaccharides, DNA, RNA, and proteins), whether attached to or released from the cell, can contribute to the development of steep chemical gradients over very short distances. It is currently difficult to predict the behavior of contaminant radionuclides and metals in such microenvironments, because the chemistry there has been difficult or impossible to define. The behavior of contaminants in such microenvironments can ultimately affect their macroscopic fates. We have successfully performed a series of U L<sub>III</sub> edge x-ray absorption fine structure (XAFS) spectroscopy, hard x-ray fluorescence (XRF) microprobe (150 nm resolution), and electron microscopy (EM) measurements on lepidocrocite thin films (~1 micron thickness) deposited on kapton films that have been inoculated with the dissimilatory metal reducing bacterium *Shewanella oneidensis* MR-1 and exposed to 0.05 mM uranyl acetate under anoxic conditions. Similarly, we have performed a series of U L<sub>III</sub> edge EXAFS measurements on lepidocrocite powders exposed to 0.05 mM uranyl acetate and exopolymeric components harvested from *S. oneidensis* MR-1 grown under aerobic conditions. These results demonstrate the utility of combining bulk XAFS with x-ray and electron microscopies.

**Keywords:** XAFS, uranium, x-ray microscopy, biogeochemistry

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## INTRODUCTION

Uranium exists in most oxidizing environments as U(VI) in the uranyl ion (UO<sub>2</sub><sup>2+</sup>), which is generally water soluble. An exception, uranyl phosphate, is typically highly insoluble. Uranium is also often found as U(IV) in highly insoluble uraninite (UO<sub>2</sub>) in reducing environments such as groundwater. Microorganisms can affect the mobility of uranium and other metals via electron transfer reactions resulting from microbial respiration. They can also affect the mobility of uranium via production of a variety of exopolymeric substances (EPS). Although EPSs may remain attached to the cell membrane (capsular material), many are freely released from the bacteria. Because they typically have a net negative

charge, exuded EPS can travel relatively unimpeded through porous media in which many mineral surfaces are negatively charged. However, some functional groups associated with EPS can also dissolve mineral surfaces, complex cations, and reincorporate the ions into solution. These processes affect the chemistry of elements that are constituents of or are sorbed to the solid phase. In addition to affecting the bioavailability of contaminant elements, the presence or absence of EPS can affect the bioavailability of macro- and micronutrients, an influence ultimately manifested in changes in the metabolic state of a microbial species.

The interactions of metal contaminants with geosurfaces and with EPS have been studied, but very little work has been done to *directly* probe the interaction of a contaminant metal with the microbe-geosurface interface. A better understanding of this

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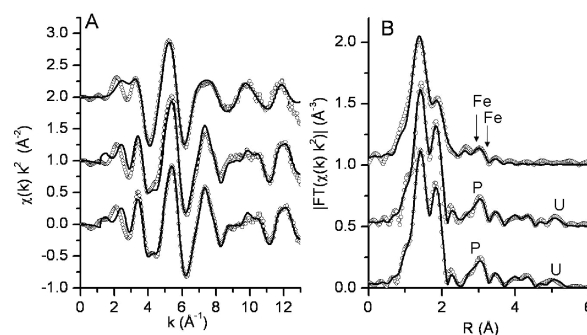
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ternary interaction will provide the information needed for more realistic models of the transport of metal and radionuclide contaminants through natural soils.

We are using x-ray absorption fine structure (XAFS) spectroscopy, x-ray fluorescence (XRF) microscopy (150 nm spatial resolution), and scanning electron microscopy (SEM) to directly probe the interactions between uranium, *Shewanella oneidensis* MR-1 (a dissimilatory metal reducing bacterium), the exopolymeric material (EPS) produced by the cell, and iron (hydr)oxides. Investigations have been performed to investigate the local chemical environment of uranium exposed to bulk lepidocrocite powders and EPS components produced under aerobic conditions. Additional studies have been made on thin films of lepidocrocite, colonized with monolayers of bacteria, and exposed to 0.05 mM uranyl acetate under anoxic conditions.

### INVESTIGATIONS OF THE ROLE OF EPS ON U-IRON (HYDR)OXIDE INTERACTIONS UNDER AEROBIC CONDITIONS

We have performed U L<sub>III</sub> edge XAFS investigations at the MRCAT [1] insertion device beam line at the Advanced Photon Source (APS) on bulk samples containing either lepidocrocite or ferrihydrite powders and U (1E-4 M) in solutions of either minimal salts bacterial growth media or EPS (i.e., the filtrate passing through a 0.2 micron filter after the onset of stationary phase growth) to directly probe the interactions between U, EPS from aerobically grown *S. oneidensis* MR-1, and iron (hydr)oxides. Measurements were made with an ion chamber filled with Ar gas at atmospheric pressure in the fluorescence mode and utilized a harmonic rejection mirror. Results from U L<sub>III</sub>-edge XAFS measurements of EPS identify P backscattering atoms around U, consistent with the presence of carboxyl and phosphoryl functional groups in the EPS (figure 1). XAFS measurements of the bulk lepidocrocite in the presence of aerobically grown EPS or growth media indicate less interaction in the solid phase between U and lepidocrocite than between U and phosphates. Further analyses of these data are consistent with some precipitation of hydrous uranyl phosphate. In contrast, XAFS measurements of bulk ferrihydrite in the presence of aerobically produced EPS indicate more U-Fe correlations in the higher coordination shells around U than with phosphoryl functional groups associated with the EPS. These XAFS results indicate that the ability of EPS to complex uranium is dependent on the iron (hydr)oxide mineral surfaces that are present in the system.

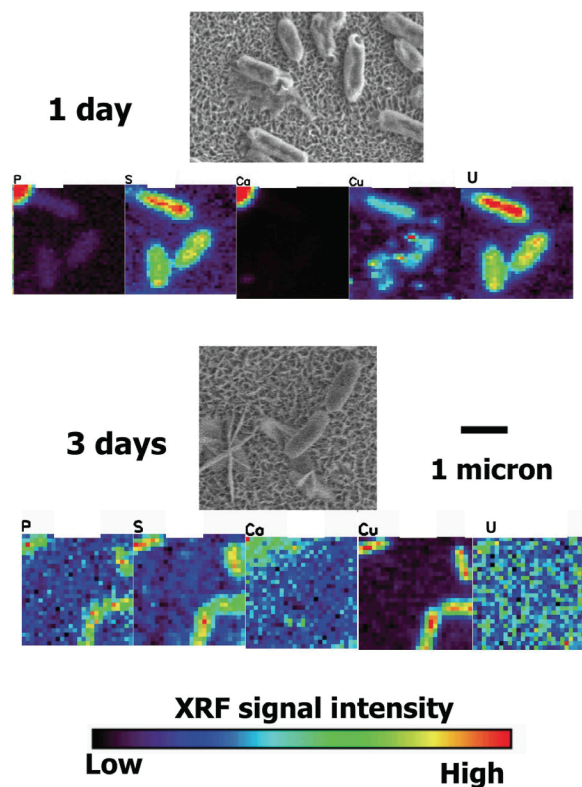


**FIGURE 1.** U L<sub>III</sub>-edge  $\chi(k)k^2$  and Fourier transformed data of U + ferrihydrite + EPS (top), U + lepidocrocite + EPS (middle), and U + EPS (bottom). See text for further details.

### INVESTIGATIONS OF URANIUM-LEPIDOCROCITE-*S. oneidensis* MR-1 INTERACTIONS UNDER ANOXIC CONDITIONS

The combination of the high brilliance of the APS synchrotron and the use of high-resolution zone plates [2] for focusing improves x-ray microimaging experiments of mineral-microbe-metal systems substantially [3]. We have used scanning electron microscopy, XRF microscopy (at the XOR 2ID-D microbe beam line at the APS), and bulk XAFS (at the MRCAT 10ID beam line at the APS) to investigate the interactions between U, *S. oneidensis* MR-1, and amorphous lepidocrocite thin films under anoxic conditions. (figure 2) Results from these studies demonstrate the ability of XRF microscopy to identify the location of U, relative to cells adhered to Fe (hydr)oxide thin films, and the ability of scanning electron microscopy (SEM) to image EPS in close association with cells adhered to the films. XRF microscopy results for samples incubated for one day indicate that U is associated with the bacteria and adjacent extracellular material. XRF microscopy results for samples incubated for three days indicate that U is dispersed throughout the biofilm and coincident with the location of EPS and biomineralization products. Results from U L<sub>III</sub>-edge XANES measurements of these films indicate that the U is not reduced after three days of incubation. Possible explanations for the lack of discernable reduction of U include (1) a lag in anaerobic respiration of the *S. oneidensis* MR-1, (2) reoxidation of the U by the predominantly ferric (hydr)oxide film, and (3) binding of the U(VI) by EPS with chemical and physical hindering of the bioreduction process. We have observed a similar lag in reduction of Fe in

fine-particle lepidocrocite. In addition, past investigations showed that poorly crystalline ferrihydrite strongly inhibited bioreduction and reoxidized U(IV) [4]. To address this issue, we have begun investigating longer incubation times, the use of different bacterial strains (*S. putrefaciens* CN32, *Geobacter* spp., and *Anaeromyxobacter* spp.), and alternative iron (hydr)oxide thin films for these experiments.



**FIGURE 2.** Scanning electron microscopy and XRF microscopy results of 1 day and 3 day incubations of *S. oneidensis* MR-1 deposited on lepidocrocite thin films deposited on kapton.

## SUMMARY

We have performed a number of x-ray-based and electron-based interrogations of mineral-metal-microbe-EPS interactions. Results indicate that integration of the strengths of each probe, relative to each other (i.e., better spatial resolution for EM, better elemental sensitivity for XRF microscopy, and better chemical sensitivity for XAFS), provide a powerful means to better understand chemical microenvironments that are critical for understanding biogeochemical transformations of contaminant metals and radionuclides.

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